Comparison of intraduodenal and intravenous glucose metabolism under clamp conditions in humans

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1Laboratory of Experimental Medicine, and Departments of 2Endocrinology and 3Gastroenterology, Hôpital Erasme, Brussels Free University, B-1070 Brussels, Belgium; and 4Institute of Physiology, School of medicine, University of Lausanne, CH-1005 Lausanne, Switzerland

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Férı, F., L. Tappy, J. Devièrë, and E. O. Balasse. Comparison of intraduodenal and intravenous glucose metabolism under clamp conditions in humans. *Am J Physiol Endocrinol Metab* 286: E176–E183, 2004. First published October 7, 2003; 10.1152/ajpendo.00201.2003.—To determine whether the uptake and metabolic partition of glucose are influenced by its delivery route, 12 normal volunteers underwent two 3-h euglycemic (~93 mg/dl) hyperinsulinemic (~43 mU/l) clamps at a 3- to 5-wk interval, one with intravenous (IV) and the other with intraduodenal (ID) glucose labeled with [3-3H]- and [U-14C]glucose. Systemic glucose was traced with [6,6-2H2]glucose in eight subjects. During the last hour of the clamps, the average glucose infusion rate (5.85 ± 0.37 vs. 5.43 ± 0.43 mg·kg⁻¹·min⁻¹; *P* = 0.02) and exogenous glucose uptake (5.66 ± 0.37 vs. 5.26 ± 0.41 mg·kg⁻¹·min⁻¹; *P* = 0.04) were borderline higher in the ID than in the IV studies. The increased uptake was entirely accounted for by increased glycolysis (H₂O production), which was attributed to the stimulation of gut metabolism by the absorptive process. No difference was observed in glucose production (ID vs. IV: 2.44 ± 0.28 vs. 2.40 ± 0.31 mg·kg⁻¹·min⁻¹) or as glucose uptake minus net glucose oxidation (2.86 ± 0.33 vs. 2.81 ± 0.35 mg·kg⁻¹·min⁻¹). Because peripheral tissues were exposed to identical glucose, insulin, and free fatty acid levels under the two experimental conditions, we assumed that glucose uptake and storage were similar during the two tests. We therefore suggest that hepatic glycogen storage (estimated as whole body minus peripheral storage) was also unaffected by the route of glucose delivery. On the other hand, in the ID tests, the glucose splanchnic extraction ratio calculated by the dual-isotope technique averaged 4.9 ± 2.3%, which is close to the figures published for IV glucose. Despite the limitations related to whole body measurements, these two sets of data do not support the idea that enteral glucose stimulates hepatic uptake more efficiently than IV glucose.

**THE OLD AND ATTRACTIVE CONCEPT** that the liver, owing to its unique position between the portal vein and systemic circulation, functions as a “filter” that retains significant amounts of glucose absorbed by the gut has been challenged for many years.

A simple and remarkable experiment conducted more than 30 years ago has greatly contributed to minimize the importance of this “anatomical privilege” of the liver in relation to postprandial glucose metabolism. McIntyre et al. (27) indeed showed in the dog that the infusion of equal amounts of glucose into the portal vein and a systemic vein produced identical peripheral levels of both glucose and insulin. This result does not in itself exclude the possibility that hepatic uptake and/or storage of glucose might have been stimulated to a greater extent during the infusion via the portal vein. This possibility is supported by the results of several subsequent animal studies (7, 18, 21, 37), but not all (2, 6, 30), in which hepatic (splanchnic) glucose metabolism was compared after oral (or intraportal) and intravenous (IV) administration. To investigate the possible mechanisms of such a stimulation in the dog, Cherrington and coworkers (see references in Ref. 8) carefully examined the interplay between the various factors controlling hepatic glucose uptake and storage. They observed that, in addition to stimulation by insulin and by the glucose load reaching the liver, hepatic glucose uptake and glycogen synthesis are also stimulated by a “portal signal” activated by a positive portal-arterial glucose concentration gradient. Although the exact nature and location of this putative sensor are not yet known, it seems to act through local neural mechanisms (1). It has been proposed that this signal allows the organism to distinguish between meal-derived and endogenously produced (or peripherally administered) glucose so as to direct the former preferentially to the liver. In one animal study (20), it was suggested that splanchnic glucose delivery results in the stimulation of net splanchnic glucose uptake whether the glucose entered the liver via the hepatic artery or via the portal vein.

In humans, relatively few authors have compared the splanchnic metabolism of oral vs. intravenous glucose. Using the hepatic vein catheterization technique, DeFronzo et al. (11) showed that, under hyperglycemic clamp conditions, glucose ingestion stimulates net splanchnic glucose uptake by a factor of six. The higher insulin levels that prevailed after glucose ingestion and the possibility that the oral load might have been incompletely absorbed within the 3 h of the study might have led to an overestimation of these results. However, on the whole, these data are clearly in favor of the operation of a portal signal in humans. On the other hand, using a dual-isotope technique, Radziuk (31, 32) found no difference between the intravenous and intraduodenal routes regarding hepatic glycogen storage. In this study, performed under nonsteady-state conditions, the intravenous glucose was infused at a rate that matched the rate of absorption of an oral glucose load, and no attempt was made to control glycemic or insulinoergic excursions.

To further explore the impact of the route of administration on glucose metabolism in humans, we recently compared the...
effects of intraduodenal and intravenous glucose infusions (13). The intraduodenal infusion has the advantage over ingestion of eliminating the unsteadiness of intestinal absorption caused by the fluctuations in gastric emptying. Using tracer methods and indirect calorimetry, we observed that, at a constant infusion rate of 6 mg·kg⁻¹·min⁻¹, the distribution of whole body glucose between glycolysis, oxidation, and storage was not affected by the route of administration (13). The significance of this comparison was, however, limited by the fact that, owing to an "increment effect," higher insulin levels and lower glucose levels were observed during the intraduodenal than in the intravenous tests. This methodological drawback was overcome in subsequent studies by Vella et al. (42), who showed that, during hyperglycemic hyperinsulinemic clamps performed under somatostatin infusion, the route of glucose administration does not affect whole body glucose uptake or hepatic glycogen synthesis, as estimated by the hepatic UDP-glucose turnover.

It is therefore still not clear whether, in humans as in the dog, the route of glucose delivery modulates the metabolic fate of glucose. The present experiments using a dual-isotope technique and indirect calorimetry extend previous investigations in the same field by comparing whole body glucose uptake, glycolysis, and storage during intraduodenal vs. intravenous glucose under glycemic and insulinemic clamp conditions.

MATERIALS AND METHODS

Protocol. Twelve healthy volunteers participated in the study. Their characteristics were the following: age, 33 ± 3 yr; sex, four females and eight males; body wt, 71 ± 3 kg, and body mass index, 23.8 ± 0.5 kg/m². The nature, purpose, and potential risks of the study were explained to the subjects, and their written informed consent was obtained before participation. The protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Brussels.

At 3- to 5-wk intervals, the subjects underwent two 3-h euglycemic hyperinsulinemic clamps, the first with intravenous (IV) glucose and the second with intraduodenal (ID) glucose. This particular sequence was chosen because knowledge of the insulin sensitivity of each subject, as estimated from the results of the IV clamp, greatly facilitates the performance of the ID clamp, which is slightly more difficult.

Around 6:00 PM on the day before the first experiment, the volunteers were injected with 20 μCi of H₂O₂ to determine total body water (TBW) volume from the H₂O concentration of basal urine and blood samples collected the next morning.

The IV clamp was performed as follows. After an overnight fast, a Teflon catheter was inserted into an antecubital vein for infusion of all test substances. Another catheter was placed in a contralateral dorsal hand vein for intermittent blood sampling, and the hand was placed in a temperature-regulated heating pad to allow arterIALIZATION of the venous blood. After a period of approximately 30 min, three basal blood samples were collected at 15-min intervals. At that time (time 0), three different infusions were started simultaneously and continued for 180 min: 1) a constant infusion of [6,6-²H₂]glucose (56 μg·kg⁻¹·min⁻¹) with a priming dose representing 35% the rate of infusion per minute (this peripheral tracer was used in only 8 of the 12 subjects studied); 2) a primed (250 mU/m²) constant infusion (25 mU/m²·min⁻¹) of insulin diluted in saline containing 3% (vol/vol) of the subject's own blood; and 3) a variable infusion of a 20% glucose solution in water containing 0.5 μCi/ml of [³H]glucose and 0.02 μCi/ml of [¹⁴C]glucose, frequently adjusted so as to maintain plasma glucose at ~90 mg/dl. Before the start of any of these infusions, the bicarbonate pool was labeled with a bolus injection of 1.5 μCi of [¹⁴C]bicarbonate. Blood samples for determination of [³H]glucose, H₂O, insulin, glucagon, and various substrates were obtained at 15-min intervals until 30 min after the end of infusion. [³H]glucose enrichment was determined only in the samples collected during the 120- to 180-min period. Samples of expired air were collected in a rubber bag at 30-min intervals for prompt analysis of [¹⁴CO₂] specific activity (SA). Timed urine specimens were obtained before and after the glucose infusion. Respiratory gas exchanges were determined by computerized open-circuit calorimetry (Deltatrac; Datex, Helsinki, Finland) during the basal period and for 15-min periods every half hour throughout the infusion.

The ID test was performed as follows. After an overnight fast, an esogastroduodenoscopy was performed using a G1F160 gastroscope (Olympus, Tokyo, Japan) to introduce a "pigtail" 6-Fr nasoduodenal drainage catheter (Cook Ireland, Limerick, Ireland), the tip of which was placed in the duodenum ~15 cm beyond the pylorus. Correct catheter positioning was confirmed fluoroscopically. The proximal end of the catheter was then passed through the nasal fossae and secured with tape on the skin next to the nostrils. The whole procedure was performed under mild sedation with 2–3 mg of intravenous midazolam (Dormicum; Hoffman-La Roche, Grenzach, Germany) and lasted for ~20 min. After subjects had rested for ~1 h, the glucose clamp was performed in exactly the same way as for the IV test, except that the labeled 20% glucose solution was administered via the duodenal catheter and contained 150 mmol/l NaCl to facilitate intestinal glucose transport (28).

Subjects were asked to make sure that the meals taken the night before each test were similar in quantity and composition and were ingested at the same hour to ensure comparable nutritional conditions. About 14 h elapsed between the last meal and the beginning of each test.

All radioactive tracers were purchased from DuPont-NEN (Boston, MA), and the [6,6-²H₂]glucose was from Campro Scientific (Veenendaal, The Netherlands).

Analytic procedures. Blood samples were collected in heparinized syringes and transferred to tubes kept on ice. The samples used to measure unlabeled and labeled glucose and lactate concentrations contained NaF, and those used to measure the glucagon concentration contained aprotinin. After centrifugation at 4°C, plasma was stored at ~2°C until assay. Plasma glucose was assayed by a glucose oxidase method (Test Combination; Boehringer, Mannheim, Germany). Plasma [³H]glucose and H₂O were measured after deproteinization by the Somogyi method. [³H]glucose was counted by dual-scintillation spectrometry on evaporated filtrates reconstituted with water, and H₂O was determined as the difference between the tritium counts obtained with and without evaporation. H₂O in plasma water was calculated by dividing its concentration in total plasma by 0.93. For the [6,6-²H₂]glucose measurements, glucose was derivatized to pentaacetyl-glucose and analyzed with GC-MS (Hewlett Packard, Palo Alto, CA) in chemical ionization mode with selective monitoring of mass-to-charge ratios 331 and 333 (39). ¹⁴CO₂ SA in expired air was determined as described previously (14).

Lactate was determined on a neutralized perchloric filtrate of plasma by a standard enzymatic method (5). Free fatty acids were assayed by an enzymatic method (NEFA; Wako, Neuss, Germany). The levels of plasma insulin (Pharmacia Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) and glucagon (glucagon RIA kit; Linco Research, St. Charles, MO) were determined by RIA. Total urinary nitrogen was assayed by the Kjeldahl method with a Kjeltec 1 apparatus (Tecator, Högås, Sweden). All measurements were made in duplicate.

Calculations. At each time point, the concentration of exogenous plasma glucose was calculated as the ratio of the [³H]glucose concentration over the [¹⁴C]glucose concentration. Kinetics and metabolic fate of exogenous glucose based on [³H]glucose, H₂O and ¹⁴CO₂ measurements. Average metabolic flux rates of exogenous glucose over a given period were calculated for the
ID and IV tests according to Eqs. 1–4, as indicated below. For the ID clamps, it was assumed that duodenally infused glucose is rapidly and totally absorbed, as we demonstrated in a previous study (13). Thus

\[ R_{a,\text{Exog G}} = I_{\text{Exog G}} - \Delta \text{pool}_{\text{Exog G}} \]  

where \( R_a \), \( I \), and \( \Delta \text{pool} \) respectively represent the average rates of disappearance, infusion, and changes in pool size of exogenous glucose (Exog G) for the period concerned. The volume of distribution of glucose was assumed to represent 130 ml/kg body wt.

\[ \text{glycolysis}_{\text{Exog G}} = \left( \Delta^n\text{H}_2\text{O in plasma water} \times TBW \right) / [\text{H}]\text{SA}_{\text{Exog G}} \]  

\[ \text{storage}_{\text{Exog G}} = R_{\text{a,Exog G}} - \text{glycolysis}_{\text{Exog G}} \]  

\[ \text{oxidation}_{\text{Exog G}} = \text{expired} [14\text{CO}_2] / [\text{C}]\text{SA}_{\text{Exog G}} \]

The oxidation data were corrected for incomplete [14CO2] recovery by using a factor of 0.54, as suggested by Schneiter et al. (34).

Total and exogenous glucose kinetics based on [1H]- and [6,6-2H2]glucose measurements. The rate of appearance (\( R_a \)) and the \( R_d \) of total glucose (exogenous + endogenous) in the systemic circulation were calculated for the 120- to 180-min period, from the measured [6,6-2H2]glucose enrichment (expressed as tracer-to-tracee ratio) by use of Steele's non-steady-state equations (38).

The \( R_a \) and \( R_d \) of exogenous glucose in the systemic circulation were determined in the same way from the calculated [3H]glucose enrichment that would prevail if exogenous glucose alone were present in the circulation.

The \( R_d \) of endogenous glucose was obtained by subtracting exogenous from total \( R_d \).

First-pass splanchnic uptake in the ID experiments was computed as the difference between the glucose infusion rate and the \( R_d \) of exogenous glucose in the systemic circulation.

Indirect calorimetry. Carbohydrate and lipid oxidation and energy expenditure were determined from CO2 production, O2 consumption, and urinary nitrogen output (15). The thermic effect of glucose was calculated as the increment of energy expenditure above baseline value. The net carbohydrate balance was calculated as the difference between the exogenous glucose disposal rate and carbohydrate oxidation, as measured by indirect calorimetry.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed using the computer program SUPERANOVA (Abacus Concepts, Berkeley, CA). Results were analyzed using a two-factor (group × time) ANOVA with repeated measures on time, and whenever a difference was detected at a statistically significant level (\( P < 0.05 \)), simultaneous pairwise comparisons between ID and IV tests were made by a modified t-test with the standard error derived from the ANOVA. Linear regression was performed by standard techniques.

RESULTS

As shown in Fig. 1, basal plasma levels of glucose, insulin, C-peptide, glucagon, and free fatty acids were similar on the IV and ID study days, but the basal lactate concentration was slightly higher in the ID study (1.04 ± 0.06 vs. 0.88 ± 0.07 mmol/l, \( P = 0.05 \)). No difference was found for basal carbohydrate oxidation or energy expenditure.

During the tests, insulin was clamped at ~43 µU/l and glucose, at ~93–95 mg/dl, i.e., a few milligrams per deciliter lower than the basal value, and there was no difference between these levels on the two study days (Fig. 1). The time courses of glucose infusion were similar, but slightly more glucose had to be infused in the ID than in the IV study (mean for the 0- to 180-min period: 4.59 ± 0.27 vs. 4.12 ± 0.26 mg·kg⁻¹·min⁻¹, \( P < 0.001 \)). Because no difference was detected in exogenous glucose concentrations, exogenous glucose uptake was also slightly (~12%) higher during the ID clamp (mean for 0–180 min: 4.24 ± 0.43 vs. 3.76 ± 0.39 mg·kg⁻¹·min⁻¹, \( P < 0.001 \)). In relation to the slight fall in glucose levels from baseline, C-peptide decreased significantly during the IV clamp but not during the ID clamp, when it remained relatively steady. During the last hour of the study period, glucagon levels were slightly but significantly higher in the ID than in the IV study (Fig. 1). Lactate levels increased in a parallel manner according to a biphasic pattern in both tests, with a maximal increase of ~0.5 mmol/l at 60 min. Their changes from baseline were not significantly different in the two tests. Free fatty acid concentrations were virtually identical in both. Energy expenditure did not change during the IV clamp but did rise very significantly during the ID test (\( P < 0.001 \)). Average carbohydrate oxidation over the 3-h clamp period was significantly higher during the ID than during the IV test (2.67 ± 0.13 vs. 2.23 ± 0.13 mg·kg⁻¹·min⁻¹, \( P < 0.001 \)).

After the glucose and insulin infusions were stopped at 180 min, a rapid fall was observed in total and exogenous glucose concentrations in both studies, but it was slightly slower in the ID tests (Fig. 1).

During the last hour of clamping, a near steady state was reached for the glucose infusion rate and exogenous glucose concentration (Fig. 1) as well as for [1H]glucose SA and [3H]glucose enrichment (Fig. 2). \(^{3}\text{H}\)O rose in a linear fashion in both tests (Fig. 2). As shown in Table 1, the rise in the exogenous glucose pool corresponded to only ~3% of the infusion rate in both clamps. Comparison of the glucose metabolism during that period in the two studies shows that, during the ID clamp, significantly higher values were observed for glucose infusion, exogenous glucose uptake, glycolysis, and oxidation (computed from [14CO2] data), total carbohydrate oxidation (estimated from gas exchange), and energy expenditure. No difference was detected for storage, whether it was measured isotopically or by indirect calorimetry (Table 1). As shown in Fig. 3, storage was strongly and positively correlated with exogenous \( R_a \) and no effect of the mode of glucose administration was detected over the whole range of glucose uptake.

In the subgroup of eight subjects whose results are shown in Table 2, the peripheral infusion of [6,6-2H2]glucose in addition to the [3-3H]glucose incorporated into the exogenous glucose solution enabled us to calculate the kinetics of total, exogenous, and endogenous glucose in the systemic circulation. The increase in the glucose infusion rate observed in the ID vs. IV experiments in the whole group of 12 subjects was no longer significant in the group of eight. The \( R_a \) in the systemic circulation for total glucose (6.44 ± 0.41 vs. 6.52 ± 0.46 mg·kg⁻¹·min⁻¹) and endogenous glucose (0.84 ± 0.08 vs. 1.10 ± 0.12 mg·kg⁻¹·min⁻¹) was not significantly different under either of the two experimental conditions.

In the ID experiments, the \( R_a \) of exogenous glucose in the systemic circulation, as determined isotopically, was only slightly (~1%) different from zero. The difference (0.30 ± 0.14 mg·kg⁻¹·min⁻¹), which represents first-pass splanchnic uptake, averaged only 4.9 ± 2.3% of the infused glucose, a value not significantly different from zero.

In the IV tests, the calculated exogenous \( R_a \) was slightly but not significantly lower than the actual amount infused (5.42 ± 0.45 vs. 5.80 ± 0.44 mg·kg⁻¹·min⁻¹).
DISCUSSION

The purpose of this study was to test whether glucose handling in humans is affected by the route of glucose administration (ID vs. IV) under clamped glucose and insulin concentrations. To mimic a postprandial situation, it would have been more appropriate to perform hyperglycemic rather than the present euglycemic hyperinsulinemic clamps. However, the strict control of insulin concentrations would have necessitated the use of somatostatin, which we wanted to avoid because it inhibits splanchnic blood flow (43) and may delay intestinal glucose absorption (23). Earlier experiments in dogs showed that systemic euglycemia does not abolish the effects of the portal signal on glucose metabolism (16).

As shown in Fig. 1, glucose and insulin concentrations were correctly clamped during the two studies. C-peptide was slightly and nonsignificantly higher in the ID tests, suggesting that some "incretin" effect occurred during the enteral infusions despite strict normoglycemia. However, any such effect was too small to influence peripheral insulin levels significantly but might have raised the insulin concentration in the portal vein. Why the glucagon levels were slightly but significantly higher during the ID clamps is not obvious. The reason may be the presence of some cross-reactivity with incretin hormones in the radioimmunoassay.

The validity of the comparison between glucose metabolism in the two studies rests on the assumption that glucose was readily and completely absorbed by the proximal portion of the duodenum. In a previous work (13), we showed that this was indeed the case for a duodenal infusion rate of 6 mg·kg⁻¹·min⁻¹, in agreement with the data published by Livesey et al. (25) evidencing fast and complete duodenal absorption for infusion rates ≤8 mg·kg⁻¹·min⁻¹. In the present study, all infusion rates (2.77–7.35 mg·kg⁻¹·min⁻¹) were below this value.

Our experimental design did not allow us to quantify the splanchnic metabolism separately, and the increase of borderline significance in exogenous Rₐ observed in ID compared with IV studies might have taken place in either splanchnic or peripheral tissues or both. Because this increased Rₐ was entirely accounted for by increased glycolysis (Table 1), the

Fig. 1. Metabolic and hormonal parameters (means ± SE) during and after intravenous (—–) and intraduodenal (—–) glucose clamps performed from 0 to 180 min. FFA, free fatty acids; CHO, carbohydrate; EE, energy expenditure; Δ, changes from baseline. Statistical significance of pairwise comparisons between the 2 tests: †P < 0.05; *P < 0.01 or less.
predominant pathway of glucose metabolism in the gut, and because the elevation of energy expenditure was observed only in the ID infusion experiments (Fig. 1 and Table 1), we favor the hypothesis that the increased exogenous $R_d$ reflects an increase in gut metabolism due to the absorptive process. In that case, our data indicate that the sum of hepatic and peripheral disposal is not significantly influenced by the route of glucose delivery. Most importantly, our data indicate unequivocally that the same conclusion applies to glucose storage. Whole body glycogen synthesis, whether measured isotopically or indirectly with the use of indirect calorimetry, was indeed linearly related to the rate of exogenous glucose uptake, and no difference due to the infusion procedure was noted (Fig. 3 and Table 1).

Because in both the ID and IV studies peripheral tissues were exposed to the same concentrations of glucose, insulin, and FFA (Fig. 1), one might at first glance conclude that their glucose consumption and storage were similar and therefore that the hepatic glucose metabolism was unaffected by the route of glucose delivery. However, this is far from certain, and no difference due to the infusion procedure was noted (Fig. 3 and Table 1).

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Table 1. Metabolic partition of exogenous glucose and indirect calorimetry data for the entire study population of 12 subjects

<table>
<thead>
<tr>
<th></th>
<th>IV</th>
<th>ID</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose infusion rate (A)</td>
<td>5.43±0.43</td>
<td>5.85±0.37</td>
<td>0.02</td>
</tr>
<tr>
<td>Δ Pool of exogenous glucose (B)</td>
<td>0.17±0.04</td>
<td>0.18±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Uptake of exogenous glucose (C = A - B)</td>
<td>5.26±0.41</td>
<td>5.66±0.37</td>
<td>0.04</td>
</tr>
<tr>
<td>Glycolysis (D)</td>
<td>2.86±0.13</td>
<td>3.22±0.17</td>
<td>0.002</td>
</tr>
<tr>
<td>Oxidation from [14]CO$_2$</td>
<td>1.71±0.10</td>
<td>1.99±0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Storage (C - D)</td>
<td>2.40±0.31</td>
<td>2.44±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Total CHO oxidation (from calorimetry) (E)</td>
<td>2.45±0.12</td>
<td>2.80±0.13</td>
<td>0.004</td>
</tr>
<tr>
<td>Net CHO balance (C - E)</td>
<td>2.81±0.35</td>
<td>2.86±0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Fat oxidation</td>
<td>0.32±0.06</td>
<td>0.28±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>14.90±0.37</td>
<td>15.68±0.11</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained during the 3rd hour (120–180 min) of the intravenous (IV) and intraduodenal (ID) glucose clamps. Data are expressed in mg/kg⁻¹·min⁻¹ except for energy expenditure, which is in kcal/kg⁻¹·min⁻¹.$ ^{10}$ P values were calculated by pairwise comparisons. NS, not significant. CHO, carbohydrate.

Table 2. Exogenous and endogenous glucose metabolism in the subgroup of 8 subjects studied by the dual-tracer method

<table>
<thead>
<tr>
<th></th>
<th>IV</th>
<th>ID</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total glucose, mg/dl</td>
<td>88±3</td>
<td>89±3</td>
<td>NS</td>
</tr>
<tr>
<td>Exogenous glucose, mg/dl</td>
<td>71±3</td>
<td>75±3</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>38±1</td>
<td>40±2</td>
<td>NS</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>69±6</td>
<td>79±4</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose fluxes, mg·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose infusion rate (A)</td>
<td>5.80±0.44</td>
<td>5.89±0.44</td>
<td>NS</td>
</tr>
<tr>
<td>$R_a$ of total glucose (B)</td>
<td>6.52±0.46</td>
<td>6.44±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>$R_a$ of exogenous glucose (C)</td>
<td>5.42±0.45</td>
<td>5.59±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>$R_a$ of endogenous glucose (B - C)</td>
<td>1.10±0.12</td>
<td>0.85±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>$R_d$ of total glucose</td>
<td>6.42±0.44</td>
<td>6.37±0.40</td>
<td>NS</td>
</tr>
<tr>
<td>First pass splanchnic uptake (A - C)</td>
<td>0.30±0.14</td>
<td>0.40±0.14</td>
<td>NS</td>
</tr>
<tr>
<td>% Splanchnic extraction [(1 - C/A) × 100]</td>
<td>4.9±2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE obtained during the 3rd hour (120–180 min) of the IV and ID glucose clamps. $R_a$, rate of appearance; $R_d$, rate of disappearance. P values were calculated by pairwise comparisons.
because experiments by Galassetti et al. (17) in dogs showed that, for identical glucose loads and identical glucose, insulin, and glucagon concentrations, intraportal as opposed to peripheral glucose infusion significantly stimulated hepatic glucose uptake and storage and inhibited nonsplanchnic (presumably muscle) glucose uptake by an amount almost identical to the amount by which it increased net hepatic glucose uptake. As a result of these opposite effects, activation of the portal signal had no impact on whole body glucose clearance. Two other studies in dogs (2, 21) also showed such a reciprocity between hepatic and peripheral responses to activation of the portal signal. However, at difference with the above-mentioned studies (17), these investigators did not include direct measurements of peripheral glucose uptake, which can be estimated only as the difference between whole body and splanchnic (hepatic) uptake. It is not known whether these observations apply to the human species, and the absence of a portal signal effect in humans remains a plausible hypothesis to account for our data. This hypothesis was recently supported by Vella et al. (42), who showed in normal volunteers that, during a hyperglycemic hyperinsulinemic clamp, enteral glucose infusion does not stimulate either whole body glucose uptake or hepatic glycogen synthesis, measured by the UDP-glucose flux, more than intravenous infusions. This latter observation is in agreement with the results of studies in which the metabolic consequences of liver transplantation were evaluated using the same parameter. Patients who have undergone orthotopic liver transplantation have a denervated liver, at least during the initial stages after transplantation and, hence, should lack the metabolic regulations secondary to the activation of portal glucose sensors. Their postprandial hepatic glycogen synthesis is similar to that observed after heart or kidney transplantation (35), but it could be argued that the greater hyperglycemic response of the liver transplant patients compensates a potential deficit in hepatic glycogen storage that would otherwise occur in these patients.

Another possible question concerning the present work is whether the hepatic glucose load and the portal-arterial glucose gradient were sufficient to activate the portal signal. Assuming that in our study the portal vein and total hepatic blood flows averaged 1,100 and 1,500 ml/min, respectively (19), and that whole blood glucose was 15% lower than plasma glucose, the hepatic glucose load during the last hour of the ID clamps can be estimated at ~23 mg/kg⁻¹·min⁻¹ (vs. ~17 mg/kg⁻¹·min⁻¹ in the IV experiments) and the portal-arterial glucose gradient at ~38 mg/dl (vs. slightly negative values in the IV tests due to glucose uptake by extrahepatic splanchnic tissues). The actual values were probably slightly lower because the estimations above assumed that uptake by the gut is minimal and that all of the glucose is absorbed as glucose, which is likely because of the almost complete recovery of duodenal glucose in the peripheral circulation. If the data obtained in dogs (8) can be extrapolated to humans, the conditions should have been fulfilled in our ID experiments for stimulating hepatic glucose uptake more strongly than in the IV studies, even under nonglycemic conditions (16). The fact that, as stated above, the portal vein insulin levels were probably slightly higher in the ID clamps should have amplified this stimulation (9). The possibility cannot of course be excluded that a larger portal-arterial glycemic gradient and/or systemic hyperglycemia are necessary to reveal the functioning of the portal signal in humans.

The participation of splanchnic tissues in glucose metabolism was evaluated in the subgroup of eight subjects explored with the double-tracer technique (Table 2). Owing to the smaller size of the group, the difference between the glucose infusion rates in the ID and IV studies was no longer significant. Endogenous glucose production rates tended to be slightly lower during the ID tests, possibly because of the higher levels of glucose (and probably of insulin) in the portal vein. During the enteral infusion, first-pass uptake averaged 4.9 ± 2.3% of administered glucose. In absolute values, the SE of this result corresponds to 0.14 mg·kg⁻¹·min⁻¹ (Table 2) i.e., <3% of infused glucose, but it amounts to 50% of the first-pass splanchnic uptake, because this uptake, measured as a difference, is quantitatively very small. Direct assessment of the extraction ratio from tracer concentration differences across the splanchnic bed is obviously the method of choice but requires hepatic vein catheterization. To our knowledge, only two studies have used this technique in humans to evaluate the metabolic fate of enteral glucose in normal volunteers. Ferrannini et al. (12) have shown that tracer-determined first-pass splanchnic extraction of oral glucose averaged 2.4 ± 0.9%, a value not significantly different from that measured in the basal state (2.7 ± 0.7%). With a different type of calculation, combining tracers and cold glucose balance data, they found that splanchnic glucose extraction ranged somewhere between 4.6 and 8.4%, a value significantly greater than basal. More recently, Basu et al. (3) found a splanchnic extraction ratio of 7.7 ± 0.6% during ID glucose infusion. Taken as a whole, these data are consistent with the 4.9 ± 2.3% observed in the present study.

It is well recognized that the measurements of net first-pass splanchnic uptake do not exactly coincide with those of hepatic uptake. First, they underestimate true hepatic uptake to the extent that the enteral glucose initially taken up by the liver is recycled after passage through glucose 6-phosphate and/or glucogen, but there is no agreement in the literature regarding the level of activity of these cycles in humans (4, 22, 33). On the other hand, splanchnic uptake overestimates hepatic uptake by an amount equivalent to the extrahepatic splanchnic uptake. To our knowledge, the latter uptake has not been quantified in humans for obvious technical reasons, but animal studies suggest that it might represent a few percent of absorbed glucose (30). Ignorance of the extent to which these two factors compensate each other adds some degree of uncertainty in the assessment of the initial hepatic trapping of duodenally administered glucose.

The dual-isotope method has been used in most attempts to evaluate the first-pass splanchnic uptake of oral glucose in humans, but, as recently reviewed (24), very discrepant results, ranging between 0 and 35%, have been reported. This scatter of the results has been attributed mainly to modeling errors, which is always a difficult issue under non-steady-state conditions. During constant ID infusions under non-steady-state glycemic conditions, Livesey et al. (25) found negligible extraction rates (2 ± 2%). On the other hand, Vella and coworkers (40, 42) found splanchnic extraction ratios as high as 10–24% in the only other available study performed in humans with intraduodenal infusions under steady-state glycemic conditions. Their results may have been overestimated as a con-
sequence of incomplete absorption due to the use of somatostatin and/or the choice of too distal an infusion site (25). To our knowledge, the present study provides the only data available in humans on first-pass splanchnic extraction of intraduodenal glucose obtained under steady glycemic and insulinemic conditions without the use of somatostatin.

A simple calculation indicates that a first-pass splanchnic sequestration of ∼5% is a metabolically plausible result. It should first be pointed out that the hepatic load (∼23 mg·kg⁻¹·min⁻¹ as calculated above) was about four times the rate of infusion (5.89 mg·kg⁻¹·min⁻¹; Table 2) because, as reported by others (25, 30), most of the glucose presented to the liver transits through the systemic circulation and only about one-quarter is newly absorbed. The absolute splanchnic uptake can thus be estimated as amounting to ∼23 × 0.05 = 1.15 mg·kg⁻¹·min⁻¹. This figure represents ∼20% of whole body glucose consumption, in agreement with the amount of glycogen known to be deposited in the liver after a glucose meal (29).

Our experimental design did not allow the calculation of splanchnic extraction of intravenous glucose for comparison with enteral glucose. The few data available in humans based on hepatic vein catheterization studies indicate that the figures for intravenously administered glucose should be ∼4–5% (10, 36), i.e., very close to the values recorded in our ID clamp experiments. This similarity could be interpreted as indicating that splanchnic glucose extraction is little affected by the route of glucose delivery. However, comparison of the two sets of data has limited value, because the two methodologies are different and because the dual-isotope method lacks precision for measuring such small fractional extractions.

In conclusion, this study shows that, under euglycemic hyperinsulinemic clamp conditions, whole body glucose uptake, glycolysis, oxidation, and storage are similar whether glucose is administered enterally or through a peripheral vein. The experimental design (double-tracer technique and indirect calorimetry) did not allow separate quantification of splanchnic (hepatic) and peripheral glucose uptake or storage. With these limitations in mind, our data do not support the idea that the rate of splanchnic glucose extraction is significantly affected by the route of glucose administration. We also confirm that first-pass splanchnic uptake is a minor mechanism for the disposal of the glucose absorbed from the gut.

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